No association between FGD1 gene polymorphisms and intellectual developmental disability in the Qinba mountain area


1Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, Institute of Population and Health, College of Life Science, Northwest University, Xian, Shaanxi, China
2Department of Pediatrics, Tangdu Hospital, The Fourth Military Medical University, Xian, Shaanxi, China
3Xian Institute of Mental Health, Xian, Shaanxi, China

*These authors contributed equally to this study.
Corresponding author: X.C. Gao
E-mail: gaoxc@nwu.edu.cn

Received November 21, 2012
Accepted April 15, 2013
Published January 10, 2014
DOI http://dx.doi.org/10.4238/2014.January.10.3

ABSTRACT. FGD1 encoding a guanine nucleotide exchange factor, specifically activates Rho GTPase cell division cycle 42 (Cdc42). Dysfunction of FGD1 causes Aarskog-Scott syndrome (MIM #305400), an X-linked disorder that may affect bone and intellectual development. However, the relationship between FGD1 and intellectual developmental disorders (IDD) remains unclear. The purpose of this study was to investigate the genetic association between the FGD1 polymorphism and IDD. Working with families from the Qinba mountain area where the occurrence of IDD is higher than the average in China, we analyzed 456 samples from 130 nuclear families, effectively controlling for stratification and environmental factors. Five SNP loci (rs2230265, rs7881608, rs2239809, rs6614244, and
rs2284710) were selected that were well distributed within the \textit{FGD1} gene. Genotyping was performed through single-strand conformation polymorphism and restriction fragment length polymorphism. The data were analyzed with transmission disequilibrium tests. In the Qinba mountain area, no significant association was observed between IDD and allele or genotype frequencies, or the haplotype of the 5 SNP loci of the \textit{FGD1} gene. The results indicate that \textit{FGD1} may not be a monogenetic X-linked factor in IDD. Further studies are required to investigate its role in intellectual development based on its specific interactions with Cdc42 or other partner proteins contributing to IDD.

\textbf{Key words:} \textit{FGD1}; Restriction fragment length polymorphism; Intellectual developmental disorders; Single nucleotide polymorphism; Single-strand conformation polymorphism

\section*{INTRODUCTION}

Various aspects of learning and memory are critically affected by synaptic plasticity (van Bokhoven, 2011), abnormality of which generally results in impaired intellectual development (Nimchinsky et al., 2002; Newey et al., 2005). Development of dendritic structures and dendritic spines is regulated by Rho guanine nucleotide exchange factors (RhoGEFs) and Rho proteins, like Cdc42, RhoA, and Rac. RhoGEFs and the Rho family of proteins are expressed in a wide range of tissues, including in brain. However, the roles of RhoGEFs and Rho proteins during mammalian development remain to be determined. In cellular events, RhoGEFs and Rho proteins regulate diverse cellular processes like organization of the actin cytoskeleton, polarization, proliferation, and differentiation.

In its diverse cellular roles, FGD1 may function in transcriptional and translational control, and in differentiation of neural and supporting cells of the nervous system. Alterations of several genes in the Rho signaling pathway have been shown to contribute to intellectual developmental disorders (IDD) (Crespi et al., 2010), such as \textit{oligophrenin-1} (Nadif Kasri et al., 2009; Pirozzi et al., 2011), \textit{ARHGEF6} (Node-Langlois et al., 2006; Ramakers et al., 2012), \textit{PAK3} (Node-Langlois et al., 2006), \textit{IL1RAP1} (Franek et al., 2011; Valnegri et al., 2011), \textit{TM4SF2} (Gomot et al., 2002; Noor et al., 2009), \textit{RSK2} (Jurkiewicz et al., 2010; Tejada et al., 2011), and \textit{FGD1} (Lebel et al., 2002; Kleefta and Hamel, 2005; Dierssen and Ramakers, 2006; Kaname et al., 2006). As reported previously, abnormalities in the \textit{FGD1} gene may lead to faciogenital dysplasia and X-linked IDD (Pasteris and Gorski, 1999). \textit{FGD1} encodes a RhoGEF that contains Dbl (DH) and pleckstrin (PH) homology domains, specifically binding to the Rho family GTPase cell division cycle 42 (Cdc42). GDP-GTP exchange of the isoprenylated form of Cdc42 can be stimulated by FGD1 (Hou et al., 2003), activating the c-Jun N-terminal kinase signaling cascade, and then regulating cell growth and differentiation. Although \textit{FGD1} mutations are primarily associated with Aarskog-Scott syndrome (Pasteris et al., 1994), the relationship between \textit{FGD1} and the accompanying phenotype of intelligence development is inconsistent with the mutant genotype: A missense mutation (P312L) in the \textit{FGD1} gene is identified as being related to non-syndromic X-linked intellectual disorders (Lebel et al., 2002). Subsequently, the results from 46 independent Aarskog-Scott syndrome
male probands from different countries show that individuals with AAS are mildly affected, experiencing learning and behavioral disabilities in early childhood (Orrico et al., 2004). Another mutation (R408Q) is the cause of attention-deficit/hyperactivity disorder (Orrico et al., 2005), which is a characteristic of IDD (Melnyk and Das, 1992). However, subjects from a large Dutch family with a mutation (R402W) at position 1204 (1204C>T) exhibit a wide spectrum of intelligence levels (Verhoeven et al., 2012).

IDD is a common intellectual and neurological disorder, characterized by an intelligence quotient (IQ) significantly lower than 70, and deficiencies in cognition and social adaption. IDD affects 2-3% of the human population. Genetic defects account for about half of IDD cases. The Qinba mountain area, which is located in Shaanxi Province, northwest China, is isolated because of poor economy and lack of transportation with an altitude from 750 to 1500 m. There the prevalence of IDD (2.78%) is higher than the average in China (1.07%), and heritability is as high as 70.23% (Li et al., 1999). The present study aimed to investigate the relationship between genetic polymorphisms of FGD1 and IDD in this area using a family-based population analysis.

MATERIAL AND METHODS

Participants

All subjects were recruited from the Qinba mountain area. Those with chromosomal and metabolic abnormality were excluded after investigation of medical history, physical examination, and the necessary clinical examination by experienced psychologists and pediatricians. Pedigree investigation and genetic sampling were carried out on IDD child and their first-degree relatives. In total, 152 children of probands (out of initially examined 7487) and their parents (304 adults) were recruited from 130 nuclear families, based upon household registration on cards. All participants gave informed consent prior to participating in this research. This project was approved by the local Ethics Committee of the National Human Genome Center.

Cognitive testing

Accurate measurements of intelligence were performed using different scales on children of different ages. Patients 4-5 years old were assessed with the Chinese Wechsler Young Children Scale of Intelligence (Gong and Dai, 1992), and children 6-14 years with the Chinese Wechsler Intelligence Scale for Children (Gong and Cai, 1993). Social adaptive behavior was evaluated with the Adaptive Scale for Infants and Children, revised by Zuo (1988). The patients were determined to have an IQ lower than 70 and social disability scores lower than 8. The adult participants were evaluated with the Chinese Classification of Mental Disorders, 2nd Revision (CCMD-2-R) (Chen, 2002).

Genotyping

Genomic DNA was extracted from whole blood (1 mL) using standard phenol/chloroform extraction methods. According to information from the National Center for Biology and
Ensemble Genome Browser, 5 loci were selected covering FGD1 using the following criteria: The locus was near or in a functional region with a >0.1 minor allele frequency among Chinese people. Primers were designed with Primer Premier 5.0. The loci, sequences, and amplification conditions are listed in Table 1. The haplotypes of 4 loci, including rs2230265, rs7881608, rs2239809, and rs6614244 were detected by single-strand conformational polymorphism-SNP, and that of rs2284710 was analyzed using restriction fragment length polymorphism.

<table>
<thead>
<tr>
<th>SNPs Primers</th>
<th>Conditions</th>
<th>Size (bp)</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2230265</td>
<td>F: 5ꞌ-GAGGACAGGGAGATGGAGG-3ꞌ R: 5ꞌ-TGACTGAGCTGGGAGGGA-3ꞌ</td>
<td>94°C-30 s, 98°C-5 min, 61°C-15 s, 72°C-30 s, 30 cycles, 72°C-5 min</td>
<td>177</td>
</tr>
<tr>
<td>rs7881608</td>
<td>F: 5ꞌ-AGCTGGGCTGGGAGGGA-3ꞌ R: 5ꞌ-TGACTGAGCTGGGAGGGA-3ꞌ</td>
<td>94°C-30 s, 95°C-5 min, 53°C-30 s, 72°C-30 s, 30 cycles, 72°C-5 min</td>
<td>218</td>
</tr>
<tr>
<td>rs2239809</td>
<td>F: 5ꞌ-TCCACACTACGCCACT-3ꞌ R: 5ꞌ-TCTCCTGACTATCCCTTCCT-3ꞌ</td>
<td>94°C-30 s, 95°C-5 min, 58.5°C-10 s, 72°C-30 s, 30 cycles, 72°C-5 min</td>
<td>183</td>
</tr>
<tr>
<td>rs6614244</td>
<td>F: 5ꞌ-GTATGTGACTATTTAGGAGG-3ꞌ R: 5ꞌ-AAGGGACTTGAGTGTTGG-3ꞌ</td>
<td>94°C-30 s, 95°C-5 min, 55.8°C-15 s, 72°C-30 s, 30 cycles, 72°C-5 min</td>
<td>195</td>
</tr>
<tr>
<td>rs2284710</td>
<td>F: 5ꞌ-AGGGACTTGAGTTGG-3ꞌ R: 5ꞌ-TCTCAGGGCTGGGGAGGGA-3ꞌ</td>
<td>94°C-30 s, 95°C-5 min, 55.8°C-10 s, 72°C-30 s, 30 cycles, 72°C-5 min</td>
<td>207</td>
</tr>
</tbody>
</table>

F = forward primer; R = reverse primer; SNPs = single nucleotide polymorphisms.

### Statistical analysis

Allele and genotype frequencies were calculated with the SPSS 17.0 software. The haplotype was analyzed with the Haploview 4.2 software. Transmission disequilibrium tests (TDT) were performed on the 130 families. Because the FGD1 gene is on the X chromosome, instead of the Y chromosome, the heterozygous mothers were counted in the statistical analysis in TDT, which were completed with UNPHASED version 3.1.3.

### RESULTS

The 5 SNP loci, distributing throughout the FGD1 gene, were selected to study the association between the FGD1 gene and IDD. There was no deviation from Hardy-Weinberg equilibrium (P > 0.05) in the genotyping frequencies. As for the TDT performed on the 130 nuclear families without genetic relationship, the results showed that the P values of the 5 SNP loci were greater than 0.05 with the heterozygous mothers included (Table 2). Because all 5 SNPs (rs2230265, rs7881608, rs2239809, rs6614244, and rs2284710) were in strong linkage disequilibrium (D’ > 0.80, r² > 0.45), a haplotype block was constructed. The haplotype analysis showed no significant difference in haplotype frequency between different groups (Table 3).
DISCUSSION

The association between the FGD1 gene and the complex disease, IDD, was investigated in a population of 456 people from 130 nuclear families in the Qinba mountain area, using a family-based method to avoid the potential confounding effects of population stratification. To the best of our knowledge, this is the first time it has been shown that there is no association between FGD1 and IDD.

During embryogenesis, FGD1 participates in membrane transportation and actin cytoskeleton rearrangement, affects brain and skeletal formation and remodeling. When it comes to brain development, mutation in FGD1 perturbs neuronal polarity and vesicular trafficking related to multiple aspects of neuronal development via signaling by Rho GTPases, and may lead to brain malformation (Bottani et al., 2007). Mutations in FGD1 are related to Aarskog-Scott syndrome, which gives rise to severe speech and cognitive deficiencies, neuropsychiatric disorders, and behavioral and learning problems (Egorov et al., 2009). To date, 27 point and 2 deletion mutations have been identified in the FGD1 gene (Orrico et al., 2007, 2010). After screening DNA from affected males, C934T, a mutation of FGD1 was reported to be responsible for Aarskog-Scott syndrome. The mutation replaced proline with leucine, destroying secondary structure. The three affected male patients presented developmental delay, characterized by severe speech and cognitive deficiencies, while the mother showed normal developmental milestones and psychosocial adjustment, a result of random inactivation of the two X chromosomes (Lebel et al., 2002). Orrico et al. (2005) also suggested that mutation of FGD1 might be implicated in patients with attention-deficit/hyperactivity disorder, and lower IQ due to inattentive, hyperactive, and impulsive conduct. However, the association between FGD1 and IDD is uncertain. In this study, the association between the FGD1 gene and IDD was studied with a family-based method based upon 130 nuclear families. The results of single-locus association were negative. Because these five loci were in strong linkage disequilibrium, a haplotype analysis was conducted. However, no association was observed in individuals or global haplotype frequencies. According to our results, the FGD1 gene may not be significantly associated with the IDD population in the Qinba mountain area.

Comparing the data we obtained with previous reports, the following might explain our results. 1) The study recruits are from a different population to that used by other researchers. The Qinba mountain region is located at the junction of 5 provinces in central and western China, including Shaanxi, Sichuan, Gansu, Hubei, and Henan Provinces. This is one of the main poverty-stricken areas in China, and people living there are isolated for high altitude and limited access. The resources available for genetic research on family linkage/association in this closed population may eliminate differences in genetic background, and avoid false-positive results and population stratification. Thus, genes other than FGD1 may be the causes of IDD in this isolated area. 2) As a complex disorder, occurrence of IDD may

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>T-U</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAAAA</td>
<td>0.404</td>
<td>35.22</td>
<td>2.98</td>
<td>0.084</td>
</tr>
<tr>
<td>CATGG</td>
<td>0.341</td>
<td>27.25</td>
<td>0.043</td>
<td>0.835</td>
</tr>
<tr>
<td>CGTAG</td>
<td>0.055</td>
<td>7.8</td>
<td>0.084</td>
<td>0.773</td>
</tr>
</tbody>
</table>

*Frequencies of two alleles among all combinations. The frequencies of these haplotypes are >0.05.
involve a variety of abnormalities in signaling pathways or in those genes that affect neural or intellectual development. Moreover, abnormalities in dendritic structures and dendritic spine morphologies are the most consistent correlates with IDD, including genetic syndromes associated with IDD, and unclassified IDD. As reported previously, abnormalities in the \textit{FGD1} gene may lead to faciogenital dysplasia and markedly variable levels of intelligence (Kaname et al., 2006). Nevertheless, low IQ might be due to inattentive, hyperactive, and impulsive conduct, which may be affected by more than the \textit{FGD1} gene. \textit{FGD1} may not be a monogenetic X-linked factor in IDD, rather it may be involved in intellectual development based on its specific interactions with Cdc42 or other partner proteins. Alternatively, \textit{FGD1} may not be significantly involved in the genetics of IDD.

**Conflicts of interest**

The authors declare no conflict of interest.

**ACKNOWLEDGMENTS**

We are thankful for the efforts of the members of the Institute of Population and Health and would like to thank all the participants and researchers who took part in this study. Research supported by the National Natural Science Foundation of China (#31371327) and the Scientific Research Program Funded by Shaanxi Provincial Education Department (#JH11241).

**REFERENCES**


A family-based association study of FGD1 and IDD


